

Agilent Bravo Automated Liquid Handling Platform: 96-Channel 250 μ L Tip Accuracy and Precision



The Agilent Bravo has nine deck positions and can be configured with interchangeable 96- or 384 disposable tip heads.

Abstract

Automation helps to scale up sample throughput without sacrificing quality. It improves accuracy (the desired dispensed volume is equal to the actual dispensed volume) and precision (a narrow distribution of dispensed volume). Automated liquid handling devices can also significantly reduce the error recurrence in a workflow.

This technical overview describes a method to measure the accuracy and precision of the Agilent Bravo in conjunction with a 96-channel LT disposable tip head at the lower end of the practical volume range (2 to 10 μ L)

Introduction

Automated liquid handling devices are used in many processes performed in laboratories today. Liquid handling automation enables larger volumes of samples to be processed, and improves accuracy (desired dispensed volume is equal to the actual dispensed volume) and precision (a narrow distribution of dispensed volume) when compared to a person operating a handheld liquid pipettor. Automated liquid handling devices can also significantly reduce the occurrence of errors in a process.¹ To further the effort to automate routine laboratory processes, Agilent Automation Solutions has developed the Agilent Bravo Automated Liquid Handling Platform.

Bravo features

The Bravo has nine deck positions which accommodate any 96-, 384-, or 1536-well Society for Biomolecular Screening (SBS) standard microplates. Deck positions can be configured with thermal cycling, heating, cooling, and shaking stations, as well as maintaining open locations for tip boxes, sample microplates, and reservoirs.

The Bravo can be used with 96- or 384-channel disposable tip heads. Heads can be interchanged in a matter of seconds. The Bravo is designed to be used either standalone or as part of a robotic laboratory automation system. It is also designed to fit inside some models of laminar flow hoods and retain laminar flow, thereby opening up cell-based plating and cellular assays to an automated liquid handling platform.

Accuracy and precision testing

This technical overview describes a method to measure the accuracy and precision of the Bravo in conjunction with a 96-channel large transfer (LT) disposable tip head at the lower end of the practical volume range (2 to 10 μL). Performance at volumes $>5 \mu\text{L}$ meets or exceeds the 10 μL performance. Measurements are determined by dispensing a tartrazine/dimethylsulfoxide (DMSO) solution into dry microplates, filling the plates with water, and measuring tartrazine absorbance. The performance of the product meets or exceeds coefficient of variance (CV) of 5% and accuracy of $\pm 10\%$ of the desired volume across the practical volume range.

Summary

The method used the Bravo 96LT head, 250 μL tips and provided the following results:

- 2 μL : 2.1% CV \pm 3.3% accuracy
- 5 μL : 1.1% CV \pm 0.3% accuracy
- 10 μL : 1.0% CV \pm 4.2% accuracy
- Greater than 10 μL : equal or better than 10 μL CV & accuracy

Materials

- Bravo with a 96-channel LT disposable tip head
- Agilent 96LT 250 μL tips (part number 19477-002)
- Agilent 96-well manual fill reservoir (part number G5498B/G#049)
- 384-well polystyrene, flat bottom plates (Greiner 781101)
- Tartrazine solution, 0.25% (w/v) dissolved in DMSO
- UV/Vis Spectrophotometer with a 405 nm filter

Results

Volume (μL)	2	5	10
Precision (% CV)	2	1	1
% Accuracy (\pm)	3.3	0.3	4.2
Outliers (5%)	6	0	0
Outliers (10%)	0	0	0
Transfer Time (seconds/plate)	22	22	37

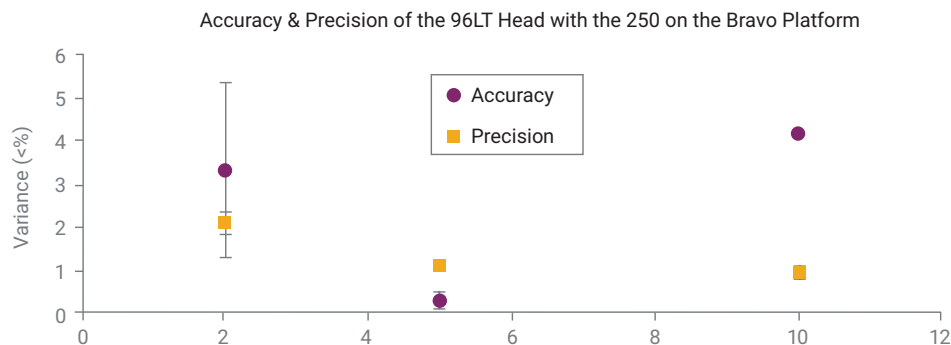


Plate compatibility

Tip type	96-Well Microplates	384-Well Microplates	1536-Well Microplates
96LT 250 μL	Yes	Yes	No
384ST 10 μL	Yes	Yes	Yes
384ST 30 μL	Yes	Yes	Yes
384ST 70 μL	Yes	Yes	No

Method

60 mL of tartrazine solution is poured into a 96-well manual fill reservoir. The reservoir is placed on position 2 of the Bravo. A 250 μL tip box is placed on position 3. A 96-well polystyrene plate is placed on position 5. Agilent VWorks automation control software liquid classes for 2, 5, and 10 μL dispenses are utilized. A VWorks protocol is created and run in the following manner:

1. Tips are pressed onto the head.
2. 2.0, 5.0, or 10.0 μL tartrazine solution is aspirated from the 96-well manual fill reservoir. Aspirate parameters are 5 mm from the bottom of the plate, and preceded by a 2 μL preaspirate volume.

3. 2.0, 5.0, or 10.0 μL tartrazine solution is dispensed into the 384-well plate. Dispense parameters are 0.2 mm from the bottom of the plate, and followed by dispensing a 2 μL blowout volume.

Dispensed volumes are diluted to 50 μL by the addition of water (in this case, with clean tips and a separate VWorks protocol). Plates are centrifuged at 1800 rpm for 60 sec to ensure full mixing and consistent well menisci. Absorbance is read at 405 nm.

Accuracy is calculated based on an equation derived from a tartrazine/DMSO calibration curve consisting of data points at 0.15, 0.32, 0.63, 1.25, 2.5, 5, 10, and 20 μL , compared to the actual absorbance value in each well.

Absorbance values in each well are used to determine the precision of the dispense. CV calculations were made by dividing the standard deviation by the mean. The accuracy and precision results are typical, based on averages of three successive plate dispenses and readings. Accuracy outliers are an average per plate. Results may vary, depending on individual experimental methods and liquid class optimization.

Conclusion

In summary, the liquid handling capabilities of the Agilent Bravo Automated Liquid Handling Platform in conjunction with a 96-channel LT disposable tip head provides precise, accurate and consistent liquid transfers. The 96-channel head can pipette across a wide volume range (1 to 250 μ L), allowing the automation of different processes using one head type. The highly configurable deck of the Bravo Automated Liquid Handling Platform along with the compact footprint makes it ideal for many applications in the laboratory including compound sample transfers, genomic applications, cell plating, and cell-based assays.

References

1. J. W. Holman, T. E. Mifflin, R. A. Felder, L. M. Demers, "Evaluation of an automated preanalytical robotic workstation at two academic health centers," Clin. Chem. 48:540-548, **2002**.

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